



Soil carbon in Australian fire-prone forests determined by climate more than fire regimes

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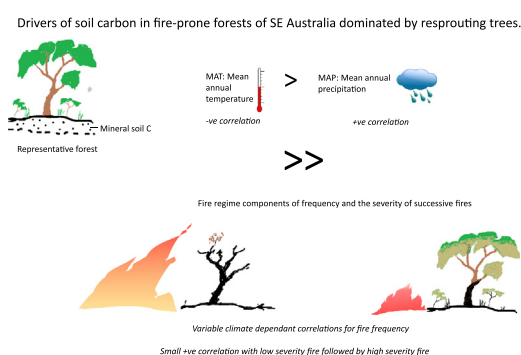
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HIGHLIGHTS

- Fire regime and climate factors considered as drivers of soil C pools.
- Lower mean annual temperature was the strongest determinant of total soil C.
- Intermediate fire frequency drove higher soil C in 2 of 3 climate scenarios.
- Higher soil C observed when low intensity fire was followed by high intensity fire.
- Recalcitrant pyrogenic C was a constant portion of the total C pool.

GRAPHICAL ABSTRACT



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ABSTRACT

Knowledge of global C cycle implications from changes to fire regime and climate are of growing importance. Studies on the role of the fire regime in combination with climate change on soil C pools are lacking. We used Bayesian modelling to estimate the soil % total C (%C_{Tot}) and % recalcitrant pyrogenic C (%RPC) from field samples collected using a stratified sampling approach. These observations were derived from the following scenarios: 1. Three fire frequencies across three distinctive climate regions in a homogeneous dry sclerophyll forest in south-eastern Australia over four decades. 2. The effects of different fire intensity combinations from successive wild-fires. We found climate had a stronger effect than fire frequency on the size of the estimated mineral soil C pool. The largest soil C pool was estimated to occur under a wet and cold (WC) climate, via presumed effects of high precipitation, an adequate growing season temperature (i.e. resulting in relatively high NPP) and winter conditions sufficiently cold to retard seasonal soil respiration rates. The smallest soil C pool was estimated in forests with lower precipitation but warmer mean annual temperature (MAT). The lower precipitation and higher temperature was likely to have retarded NPP and litter decomposition rates but may have had little effect on relative soil respiration. Small effects associated with fire frequency were found, but both their magnitude and direction were climate dependent. There was an increase in soil C associated with a low intensity fire being followed by a high intensity fire. For both fire frequency and intensity the response of %RPC mirrored that of %C_{Tot}: i.e. it was effectively a constant across all combinations of climate and fire regimes sampled.

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1. Introduction

Fires have major, immediate and longer-term effects on the turnover and make-up of organic carbon (C) via heating and consumption of

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biomass and the redistribution of residual, oxidised solids and gasses into the atmosphere and soil. There is fossil evidence of vegetation fires dating back to the Silurian (ca. 420 Mya), a time when vegetation cover and atmospheric oxygen concentrations became sufficient to sustain combustion (Glasspool et al., 2004; Jones and Chaloner, 1991). Rapid oxidation of organic matter (living and dead) facilitated by fire can release gasses directly to the atmosphere while the fate of non-gaseous pyrogenic products is more variable (Lal, 2013; Mouillot et al., 2006). This is determined by the composition and size of non-gaseous products, coupled with the timing and strength of dispersal forces applied to them (Santín et al., 2016; Lal, 2013). In some forests, very little soil organic matter (SOM) is directly altered by fire, as heating in soils under moving fires is limited to the near surface (i.e. 0 to 5 cm depth) (Mondal and Sukumar, 2014; Bradstock and Auld, 1995; Raison et al., 1986). The degree of consumption of the overlying humus is determined by its moisture content and the intensity of fire at the time, both of these factors being partially driven by weather (Santín et al., 2016; Boby et al., 2010; Bradstock et al., 2010). Fires leave a spectrum of biologically fixed C in the landscape ranging from living plant tissue to fully charred material and soots.

The portion of biologically fixed C in the post-fire landscape consisting of partially to fully charred plant tissue and soots, is termed pyrogenic carbon, PyC (Jenkins et al., 2016). Substantial research effort has been expended on the perceived C sequestration potential of PyC associated with its assumed recalcitrance (Baldock et al., 2013; Krull et al., 2003; Skjemstad et al., 1998). PyC recalcitrance can be strong, with observed persistence for centennial and millennial timescales, but is dependent on formation at high temperatures (>750 °C) from coarse, lignified fuels (Bird et al., 2015; Bodí et al., 2014; Hammes et al., 2007). Decomposition rates in chars formed at lower temperatures (<450 °C) or from fine material (low lignin content) may have weaker recalcitrance, which in some cases may be similar to other SOM particles of the same dimensions (Norwood et al., 2013; Knicker, 2011; Hammes et al., 2007). The recalcitrance of all SOM, including PyC, is enhanced when incorporated into the mineral soil where it is shielded from oxidation and other chemically degrading processes associated with fire (Santín et al., 2016). It has been argued that this physical protection by location in the soil is potentially more important to C mean residence time than the chemical recalcitrance of the specific SOM under consideration (Lal, 2013).

In natural forests, woodlands and shrublands, estimates of mineral soil C pools can exceed the aboveground amount, emphasising its importance in the global C cycle (Santín and Doerr, 2016; Vargas et al., 2008; Beringer et al., 2007). Despite the potential importance of soil pools of C, little is known about the way these pools may respond to variations in fire regimes (i.e. the frequency, intensity and season of fires) (Keeley et al., 2012). Such knowledge is important given that many forests in differing parts of the world are fire-prone (Archibald et al., 2013; Keeley et al., 2012), and that future changes in fire regimes are predicted (Clarke et al., 2011; Flannigan et al., 2009). A fundamental understanding of how soil C pools are affected by fire regimes is needed to predict their long-term fate given anthropogenic global change.

This study is an examination of how variations in recent fire regimes in fire-prone *Eucalyptus* dominated forests, in south-eastern Australia, have affected soil pools of C and recalcitrant pyrogenic C (RPC; the most recalcitrant fraction of PyC). This study also examines how such fire regime effects may be amended by regional-scale variations in temperature and rainfall within a homogenous forest type. Climatic models suggest that temperatures are likely to increase across eucalypt dominated forests, with possible increases or decreases in precipitation at a local level (CSIRO and Bureau of Meteorology, 2015; Clarke et al., 2013; Clarke et al., 2011). Such shifts have the potential to change net primary productivity (NPP), fire-proneness of the vegetation through floristic shifts and the incidence of severe fire weather in the landscape (Bradstock et al., 2014; Flannigan et al., 2009; Roxburgh et al., 2005). This study was designed to provide some integrated insight into the

way that changes to fire regimes and climate may affect: i) soil C pools generally; and ii) the ability to enhance long-term C sequestration in soils through manipulation of the fire regime using planned burning. For example, Adams (2013) and Volkova and Weston (2015) have proposed that planned burning could be used to enhance soil pools of PyC, though the evidence for appreciable inputs from such fires is equivocal (Jenkins et al., 2016).

This study focussed on the fire frequency and sequential fire intensity aspects of the fire regime. Given evidence that C pools in the soil may vary as a function of time since fire (Sawyer et al., 2018) the study design held this effect constant by sampling only sites burnt 10 to 12 years previously. Over this time since fire interval, soil C pools were found to be stable after both unplanned and planned fires (Sawyer et al., 2018). The study was carried out across the Sydney Basin Bioregion, which is dominated by fire-prone eucalypt forests. Variations in fire frequency created by both unplanned and planned fires were used to explore effects on soil C pools at sites distributed across rainfall and temperature gradients. Remotely sensed estimates of fire severity (estimated damage to vegetation from fire) were used as a proxy of fire intensity in the sampling design.

The study therefore aimed to estimate how fire frequency and intensity combinations affected the distribution of total C and RPC in mineral soils across regional-scale variations in climate (i.e. mean annual precipitation and temperature).

2. Methods

2.1. Study sites

The study was carried out in the Sydney Basin Bioregion in south-eastern Australia (Fig. 1). The dissected landscapes of this Bioregion are dominated by sedimentary geology of mainly Permian/Triassic origins that result in predominantly acidic (e.g. pH 4 to 4.5), shallow (ca. 20 to 30 cm), sandy soils. Climate is cool-warm temperate with aseasonal rainfall. Vegetation is predominantly open forest and woodland (i.e. dry sclerophyll forest (DSF), (Keith, 2004)), dominated by *Eucalyptus*, *Corymbia* and *Angophora* spp., with an understorey often rich in sclerophyllous shrub species, graminoids and sedges (Keith, 2004). The region is fire-prone with an average interval of 15 to 20 years between fires (Bradstock et al., 2012).

The sites sampled for this study were a subset of those originally established to examine vegetation responses to climatic and fire regime gradients across the Bioregion (Hammill et al., 2016). Areas in several parts of the Bioregion characterized by contrasting combinations of mean annual precipitation (MAP) and mean annual temperature (MAT), were initially identified, in order to test the effects of variations in rainfall and temperature on soil C. Two areas with MAP of 1200 mm and contrasting MATs (12 °C, 16 °C) were classified as wet and cold (WC) and wet and warm (WW) respectively. A third area, with MAP of 1000 mm and MAT of 16 °C, was classified as intermediate and warm (IW) (Fig. 1). Study sites were then selected within these climatically differentiated areas (hereafter referred to as climatic regions) based on fire regime attributes (i.e. fire frequency and fire intensity) in order to test effects of fire regimes on soil C.

Three levels of fire frequency (FF; one, two or four fires between 1972 and 2003) were selected for each combination of MAT and MAP. These selections were based on mapped fire records since 1972 (New South Wales Office of Environment and Heritage unpublished data). Three replicate sites for each combination of MAT, MAP and FF class were sampled. All sites were situated in areas last burnt in the 2001/2002 or 2002/2003 peak fire seasons (i.e. likelihood of similar intensity). Sampling was conducted over a 2.5 year period (2011–2013), therefore equating to a time since fire range of 10–12 years. We assumed there were no major biases in fire regimes among the study sites prior to 1972.

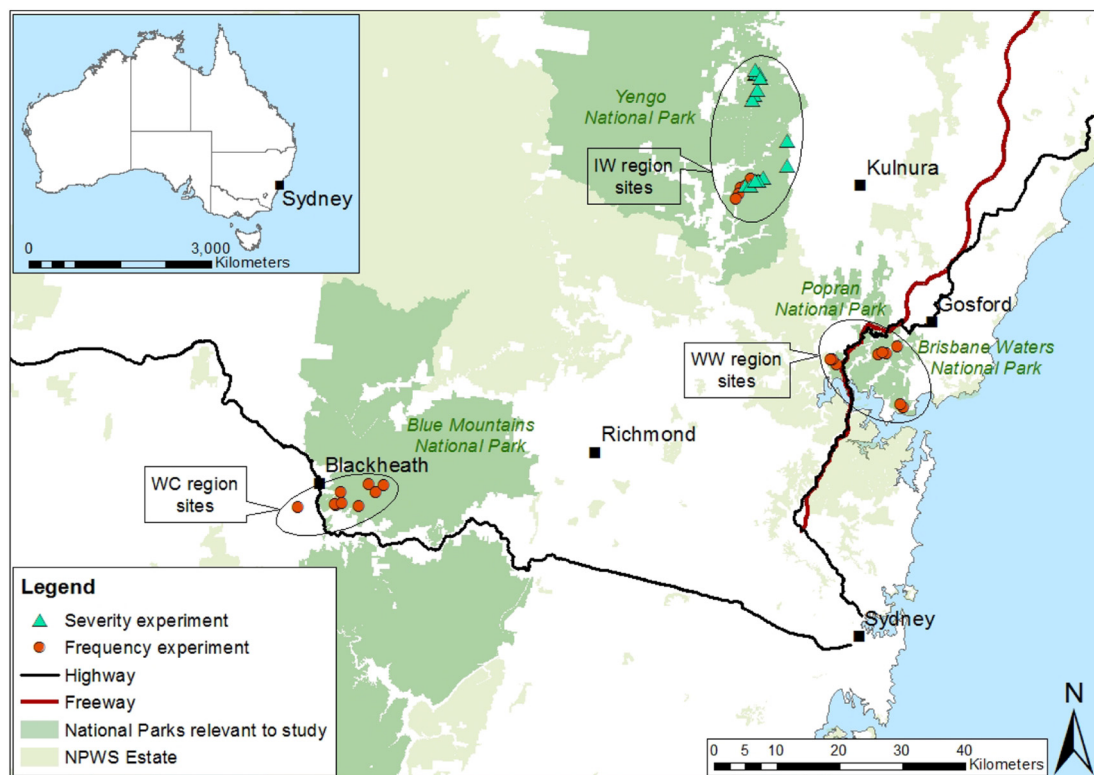


Fig. 1. Map of fire frequency and fire intensity study sites with insert locating Sydney region within Australia. Sites are grouped within the climatic regions indicated: wet and cold (WC); intermediate and warm (IW); and wet and warm (WW).

A separate investigation of the effects of fire intensity on soil C was carried out in one climatic region (IW, Fig. 1) at sites burnt by 2 successive fires (1993/1994, 2001/2002 fire seasons). Indices of fire intensity were derived by estimating fire severity from remote sensing, using methods described by Hammill and Bradstock (2006). For both fire seasons, areas that were burnt at either high severity which infers high intensity (H, canopies completely scorched or consumed) or low severity which infers low intensity (L, fire confined to the understorey) (Hammill and Bradstock, 2006) were identified. Sites with combinations of successive high/high (HH), high/low (HL), low/high (LH) and low/low (LL) were then identified and sampled. Between three and five replicate sites for each combination were sampled. It was only possible to obtain sufficient quality fire severity data at potentially accessible sites for this one climatic region.

All sites sampled in both studies (FF and intensity combinations) were situated in DSF on sandstone derived soils, on ridge tops with slopes of $<10^\circ$. To minimise the effects of spatial correlation and pseudo replication, sites within each experimental category (climate region, fire frequency and fire intensity combination) were separated by the maximum extent achievable (within practical constraints) with the minimum separation being approximately 500 m.

2.2. Field sampling

In natural forests and woodlands SOM distribution exhibits substantial spatial heterogeneity (García-Palacios et al., 2011; Liu et al., 2011; Fraterrigo et al., 2005). This spatial heterogeneity creates difficulties and expense in experimental design and analysis, in contrast to agricultural systems. There is, however, a degree of predictability in the distribution of C and other biologically cycled elements associated with the degree of concentration in biological activity, e.g. soil horizon, and the proximity to trees (Gallardo, 2003; Stoyan et al., 2000). This study utilised this concept in a stratified approach to sampling that was

aimed at accounting for some of this heterogeneity. It was assumed that SOM distribution was a function of soil depth, proximity to trees and variations in tree bark type.

The dominant *Eucalyptus*, *Corymbia* and *Angophora* species in the forests of the study area vary widely in their bark characteristics (Brooker et al., 2012). Some species commonly found across the region (e.g. *E. rossi*, *A. costata*) have smooth bark which is shed annually, whereas other common species have rough bark that is either tightly bound (e.g. *E. sieberii*) or loose and stringy (*E. globoidea*) but not regularly shed. Thus, the area surrounding the base of trees of species with contrasting bark types is subject to characteristically different patterns of bark accumulation, thereby creating fine-scale heterogeneity in potential C inputs to the soil during and after combustion. In each site all litter and any duff were carefully removed to expose the surface of the mineral soil prior to soil collection. Mineral soils were then sampled in two depth classes (0 to 5 cm and 5 to 15 cm) from the same core hole and in three micro-sites based on proximity to trees and their bark type. These were: Rough, adjacent to base of rough barked trees; Smooth, adjacent to base of smooth barked trees; and Open, patches largely between tree crowns.

Within each of these 6 sampling combinations, 4 replicate cores were collected, pooled and processed in accordance with the method described in Sawyer et al. (2018).

2.3. Soil total C assay

Prior to this assay a randomly selected subgroup of soil samples was subjected to the hydrochloric acid fizz test to detect the presence of inorganic C. Significant contributions from inorganic C were very unlikely in this soil type and this was confirmed by the lack of reactivity to the acid test. Analysis of the soil for its total C concentration was conducted using a LECO dry combustion spectrometer (1250 °C) with results expressed as % mass of soil (% C_{Tot}).

2.4. Recalcitrant pyrogenic C assay

A modified 1N nitric acid (HNO_3) and 35% hydrogen peroxide (H_2O_2) digest (KMD) method was used (Kurth et al., 2006) to remove the biologically and chemically degradable C, leaving the most recalcitrant pyrogenic C (RPC). Details of the modifications and digest method used have been previously published (Sawyer et al., 2018). In brief the method involved:

- i) oven drying of samples to constant weight at 45 °C;
- ii) pre-grinding of samples in a ball mill (TEMA brand) to pass through 120 μm sieve;
- iii) use of reflux tubes capped with teardrop condensers to control moisture loss (Licata and Sanford, 2012);
- iv) use of a block digester with a pre-programmed temperature control unit to slowly ramp up (8 h) to the operating temperature of 100 °C for a duration of 16 h, helping to control volatility;
- v) samples recovered by evaporation in drying ovens (85 °C) after decanting and washing the oxidising reagents away;
- vi) recovered digested soil samples assayed for total C as % mass of soil, using a dry combustion spectrometer at 1250 °C;
- vii) scanning electron microscopy to examine a random selection of post-digest examples to ensure all remaining identifiable organic matter had the appearance of charcoal;
- viii) a range of standard mixes of chemically clean silicious sand with varying known amounts of charcoal, sawdust and humic acid were digested and underwent subsequent C assay. Effectively, all non-charcoal organic matter was consumed by the digest while approximately 60% of the charcoal C survived.

2.5. Statistical analyses

We employed Bayesian modelling to directly estimate differences in soil C components (% C_{Tot} and % RPC) between fire regime and climatic combinations, while explicitly accounting for all sources of uncertainty. Conventional hypothesis testing, such as analysis of variance, would be unable to detect subtle effects against a background of substantial natural variability. By using this Bayesian approach we were able to address variation in replicate measures of pyrogenic carbon, and to apply variable selection (akin to ensemble modelling) within a single, consistent analytical framework. This builds on the Bayesian modelling approach used by Leon and Gonzalez (2009) for prediction of C content of Scottish soils.

The analysis consisted of a Bayesian multiple linear regression model incorporating variable selection and fitted using Markov Chain Monte Carlo (MCMC) methods. The approach is similar to that referred to as Bayesian Variable Selection Regression by Guan and Stephens (2011) and to Indicator Model Selection by O'Hara and Sillanpää (2009). The choice of this approach was motivated by: i) recognition of the scarcity in knowledge of the relationship between soil C and fire regimes, which makes choosing an *a priori* candidate model impractical; ii) avoidance of arbitrary model reduction and selection (e.g. AIC step-wise methods) that may discard possibly useful alternative models; iii) the ability to generate predictions on the basis of all candidate models identified by the fitting process, in a manner similar to model averaging or ensemble models; and iv) the ability to report on effect sizes and probabilities in direct terms and without requiring corrections for multiple comparisons. Model fitting was done using the JAGS software via the rjags package for R (Plummer et al., 2016). For the full R and JAGS code see: https://github.com/mbedward/robert_fire_freq/tree/sawyer_2018b and https://github.com/mbedward/robert_fire_sev/tree/sawyer_2018b.

2.5.1. Model description

A two-step modelling process was used in the investigation of fire frequency and fire intensity effects. The first step was to estimate %

C_{Tot} , then using the output as an input to the second step, % RPC estimation. This enabled examination of the relationship between % RPC and % C_{Tot} and allowed discrimination of effects of fire regime, region, micro-site and soil depth on % RPC that were additional to any such influences on % C_{Tot} .

2.5.1.1. Relative importance of fire frequency, intensity, region and site characteristics on % C_{Tot} . Ensemble modelling was used initially to derive predicted means of % C_{Tot} from log-transformed % C empirical data, as a function of fire frequency across all factors (soil depth, region, micro-site). Regression analysis was performed, ranking the importance of variables by inclusion rate, assigning 95% credible intervals, determining whether the 95% credible intervals included zero, and indicating the direction of any non-zero effect. A similar approach was then used to derive predicted means of % C_{Tot} for each fire intensity combination, soil depth and micro-site.

2.5.1.2. Relative importance of fire frequency, intensity, region, site characteristics and % C_{Tot} on RPC. For the second step, ensemble modelling estimates for mean % RPC as a function of either fire frequency or fire intensity, across all relevant factors (soil depth, region, micro-site, % C_{Tot}), were generated using the same modelling process described above. Within each combination, mean %RPC was calculated for each field sample by fitting a heavy-tailed distribution (a t-distribution with unknown mean, standard deviation and shape parameter) to the replicate RPC assay values. This approach ensured that any outlying assay values would be down-weighted in a data-driven manner. The mean of the fitted distribution for each experimental category became the response value for that MCMC iteration.

3. Results

3.1. Relative importance of fire frequency, climatic region and site characteristics on % C_{Tot}

The predicted mean concentration of C (% C_{Tot}) was <6.5% in all categories (Fig. 2). % C_{Tot} was consistently higher in the upper soil layer (0 to 5 cm) compared with the lower soil layer (5 to 15 cm, Fig. 2). The patterns of differences among the other predictors were also strongest in the upper soil layer where % C_{Tot} tended to be higher for two fires, compared with one or four fires in the IW and WC regions (Fig. 2), though the 95% credible intervals were large. This pattern was particularly evident in micro-sites beneath trees (Rough and Smooth barked micro-sites, Fig. 2), but less apparent in the micro-site in gaps between trees (Open, Fig. 2). In the WW region, there was a trend for higher % C_{Tot} with four fires (FF4) (Fig. 2) but the inclusion rate for this effect was low (Appendix A, Table A.1) and 95% credible intervals were large. This pattern was generally repeated in the lower soil layer (5 to 15 cm) in the respective regions, though differences between micro-sites were more subdued. In general, the Open micro-site had lower % C_{Tot} than the micro-sites positioned beneath trees (Fig. 2).

The strong inclusion rates in the ensemble modelling for interactions between fire frequency and region (ca. 39% to 91%, Appendix A, Table A.1) and the three-way interactions between fire frequency, region and micro-site (ca. 15% to 47%, Appendix A, Table A.1) further support the patterns described. Three-way interactions between fire frequency, region and soil depth had inclusion rates ranging from ca. 13% to 31% (Appendix A, Table A.1).

3.2. Relative importance of fire intensity and site characteristics on % C_{Tot}

The % C_{Tot} in all fire intensity categories, across all site characteristics, was less than ca. 4.1% by weight, based on the ensemble modelling (Fig. 3). The % C_{Tot} for the upper soil layer (0 to 5 cm) was consistently greater than the lower soil layer (5 to 15 cm) with a mean difference of 1.57% when all other factors were combined. The pattern of difference

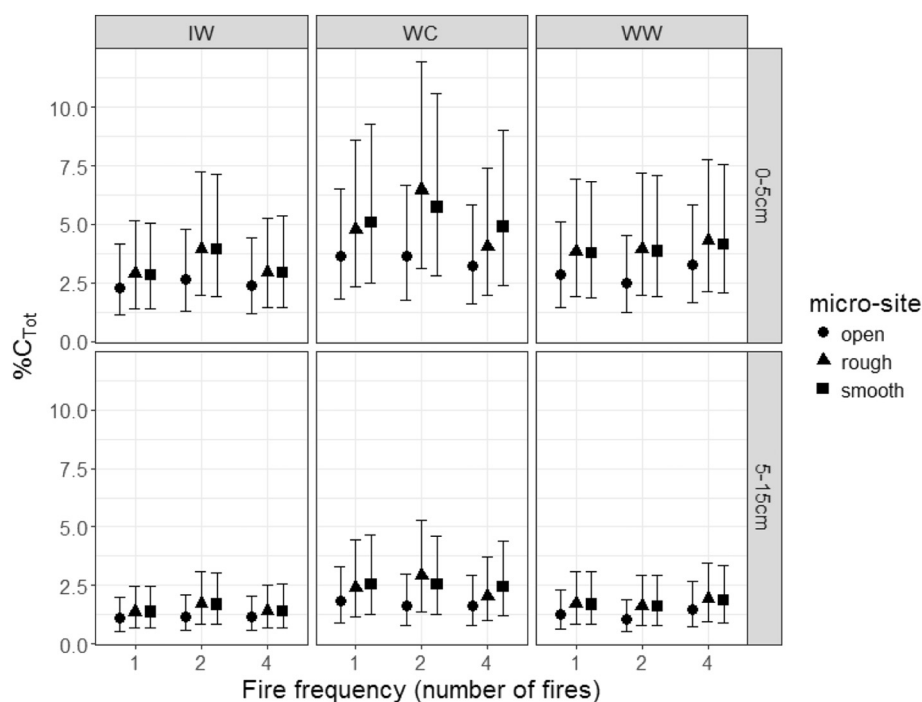


Fig. 2. Predicted mean total %C as % mass of soil (with upper and lower 95% credible intervals) from ensemble modelling categorized by fire frequency, region and site characteristics. Fire frequency was represented as the number of fires (1,2,4) over 30 years prior to sampling 10 years post fire. Regions were characterized as: wet, cool (WC), annual precipitation 1200 mm and annual average temperature 12 °C; wet, warm (WW) annual precipitation 1200 mm, annual average temperature 16 °C; intermediate precipitation, warm (IW) annual precipitation 1000 mm, annual average temperature 16 °C. Soil depth was characterized by two layers (0–5 cm; 5–15 cm). Micro-sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open).

for the other predictors, i.e. intensity combination and micro-sites, was strongest in the upper soil layer as reflected by their two-way interaction inclusion rates (ca. 24% to 50%, [Appendix A, Table A.2](#)).

The combination of low fire intensity followed by high (LH) had greater C concentrations than those predicted for other combinations

across all micro-sites, though the 95% credible intervals were large. The differences for % C_{Tot} between the other intensity combinations were relatively small. However, the order of the remaining intensity combinations for % C_{Tot} showed variation between the micro-sites ([Fig. 3](#)). Within any one fire intensity combination the % C_{Tot} was

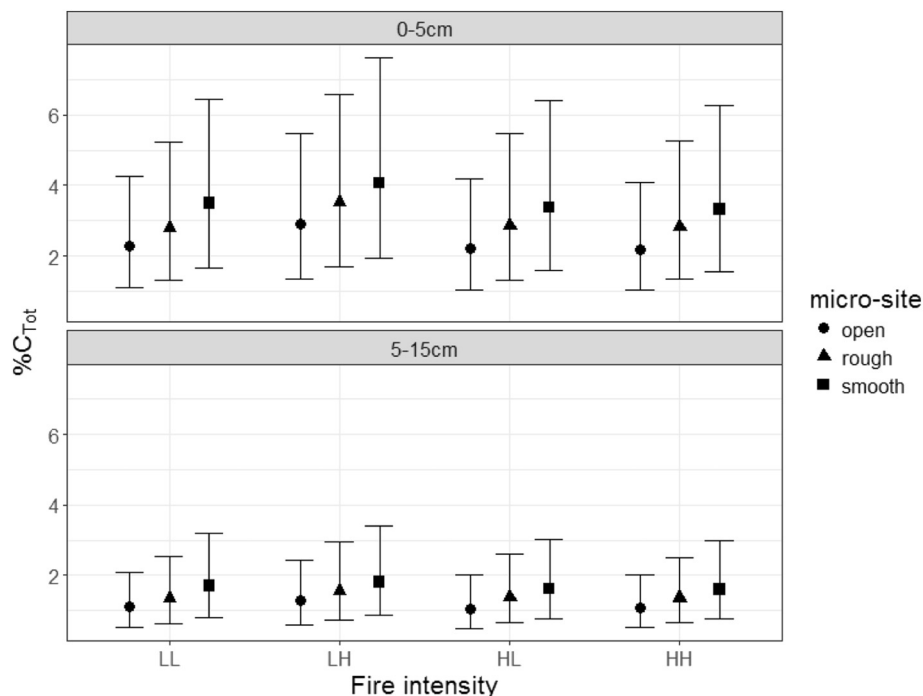


Fig. 3. Predicted mean total %C as % mass of soil (with upper and lower 95% credible intervals) from ensemble modelling categorized by fire intensity and site characteristics. Fire intensity was rated low (L), tree crowns unaffected, or high (H), tree crowns affected. Sites selected from two successive fires separated by 8 years (soil collected 10 years post the last fire) yielding four intensity combinations (LL, LH, HL, HH). Soil depth was characterized by two layers (0–5 cm; 5–15 cm). Micro-sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open).

highest under trees with shedding bark (Smooth) followed by under trees with rough bark (Rough) and lowest in micro-sites between trees (Open). These patterns applied to both soil layers, though in the lower soil layer (5 to 15 cm) differences were more subdued.

3.3. Relative importance of fire frequency, region and site characteristics on recalcitrant pyrogenic C

Predicted mean recalcitrant pyrogenic C concentration in the soil (% by weight; % RPC) was generally low (<1%) irrespective of fire history, region and site effects (Fig. 4). The strongest predictor of % RPC was % C_{Tot} with 100% inclusion rate in the ensemble modelling (Appendix A, Table A.3). Soil depth 5 to 15 cm and four fires (FF4) were the only predictors of mean % RPC to have inclusion rates above 5% (ca. 13% to 16.5%, respectively, Appendix A, Table A.3) in the ensemble modelling.

Effects of region and micro-site were considerably less influential with low inclusion rates (ca. 4% and 1% respectively, Appendix A, Table A.3). Interactions between these predictors had little influence based on very low inclusion rates in the ensemble modelling (i.e. <1%, Appendix A, Table A.3). Therefore, the distribution of % RPC effectively mirrored that for % C_{Tot} across all categories (Figs. 2 and 4): i.e. % RPC was effectively a constant fraction of % C_{Tot} across all categories (Appendix B, Fig. B.1).

3.4. Relative importance of fire intensity and site characteristics on % RPC

% RPC was <0.65% in all categories (Fig. 5). The strongest predictor of % RPC was % C_{Tot} with 100% inclusion rate in the ensemble modelling (Appendix A, Table A.4). The fire intensity and micro-site effects on % RPC were negligible, with the highest inclusion rate for any factor being <5%. The inclusion rates for interactions quickly declined to zero (Appendix A, Table A.4). Effectively the pattern of distribution for % RPC mirrored that established for % C_{Tot} (Figs. 3 and 5): i.e. % RPC was effectively a constant fraction of % C_{Tot} across all categories (Appendix B, Fig. B.2).

4. Discussion

The results indicated that % RPC generally remained constant as a proportion of % C_{Tot} across fire regime, climate and site characteristic categories (Appendix B). Similarly, Wang et al. (2018) found that total soil organic carbon (SOC) was the strongest predictor of PyC across a range of environments in south-eastern Australia. The consistency of this conversion rate of C to this recalcitrant form is important when considering both the measurement and the manipulation of soil C for sequestration. Effects of fire regime and climatic variation on mineral soil C were therefore registered principally through responses of % C_{Tot} rather than % RPC.

Climatic region was a much stronger determinant of % C_{Tot} pools than any of the fire regime components (i.e. frequency and intensity) examined in this study. The highest % C_{Tot} was estimated for the WC region. In both of the other regions the % C_{Tot} levels were substantially lower than WC, with IW being the lowest. The detectability of the fire regime effect was small, and its direction driven by climatic factors. The role of climate is illustrated by the peak in % C_{Tot} associated with the intermediate fire frequency treatment (two fires over 40 years) in the WC and IW regions, contrasted by little variation in % C_{Tot} across the three fire frequency treatments in the WW region. This has important implications and needs careful interpretation when evaluating fire regime management impacts on C under various climate change scenarios. In the fire intensity study, the peak of % C_{Tot} was observed in LH. Notably this work was confined to the one climatic region, IW, which had the lowest % C_{Tot} of the three regions.

The higher % C_{Tot} in the WC region was associated with lower MAT (driven by cold winter minimums), which may be sufficient to retard heterotrophic soil respiration rates, while higher MAP and adequate growing season temperatures may support strong NPP. The difference in soil C between this cool temperate forest region and the warm temperate counterparts on similar geology (i.e., the WW, IW) was within the range expected from the modelling of Haverd et al. (2013). This work also supports the NPP/heterotrophic soil respiration mechanism

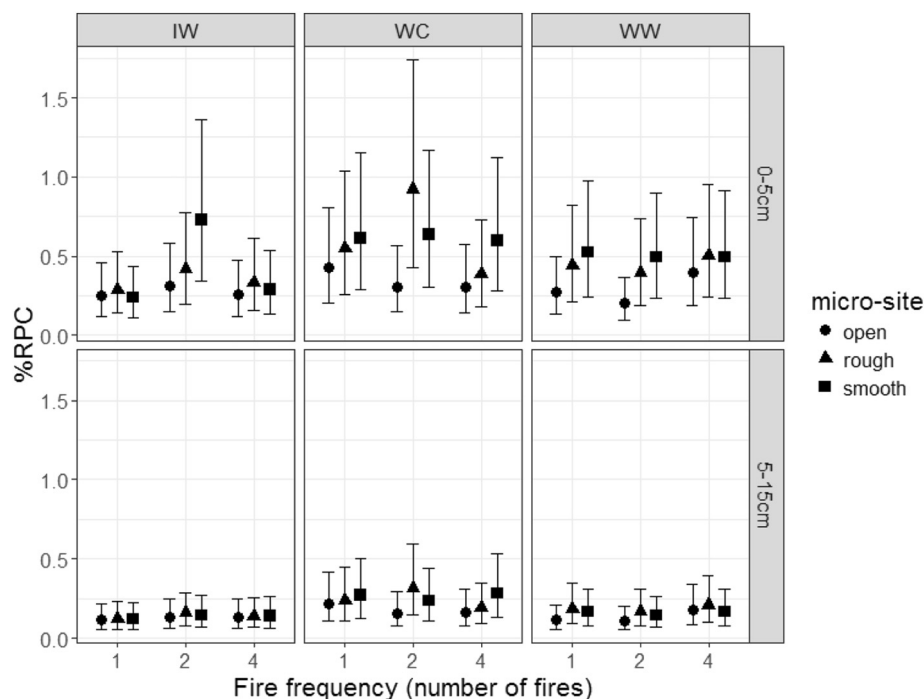


Fig. 4. Predicted mean % RPC as % mass of soil (with upper and lower 95% credible intervals) from ensemble modelling categorized by fire frequency, region and site characteristics. Fire frequency was represented as the number of fires (1,2,4) over 30 years prior to sampling 10 years post fire. Regions were characterized as: wet, cool (WC), annual precipitation 1200 mm and annual average temperature 12 °C; wet, warm (WW) annual precipitation 1200 mm, annual average temperature 16 °C; intermediate precipitation, warm (IW) annual precipitation 1000 mm, annual average temperature 16 °C. Soil depth was characterized by two layers (0–5 cm; 5–15 cm). Micro-sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open).

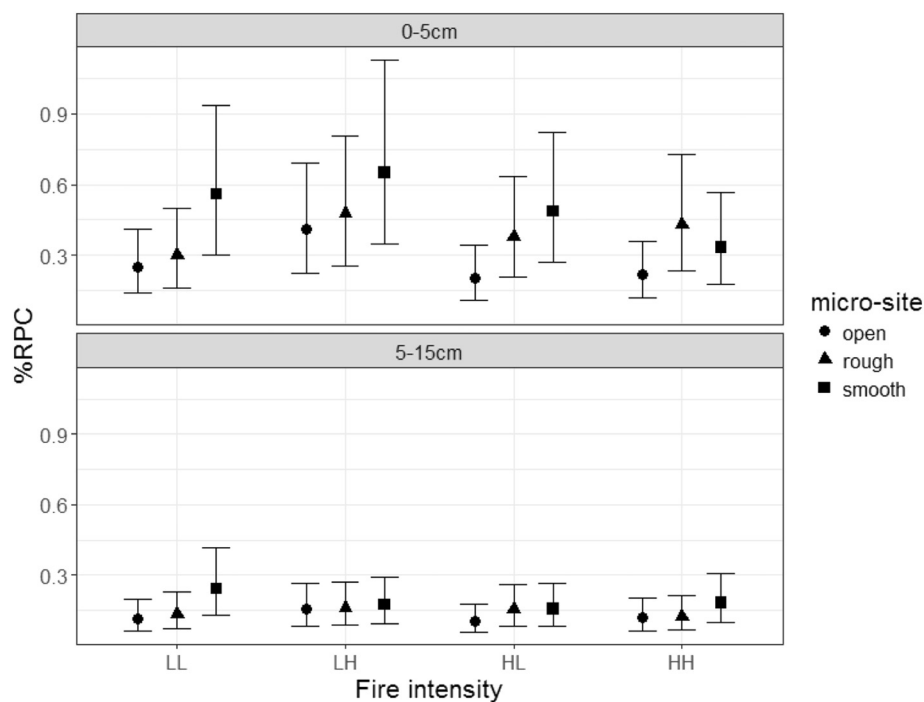


Fig. 5. Predicted mean % RPC as % mass of soil (with upper and lower 95% credible intervals) from ensemble modelling categorized by fire intensity and site characteristics. Fire intensity was rated low (L), tree crowns unaffected, or high (H), tree crowns affected. Sites selected from two successive fires separated by 8 years (soil collected 10 years post fire) yielding four intensity combinations (LL, LH, HL, HH). Soil depth was characterized by two layers (0–5 cm; 5–15 cm). Micro-sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open).

we proposed, with longer soil C residence time in the cool temperate versus warm temperate region (inferred lower soil microbial respiration). Similarly, Thomas et al. (2014) describe climatic trends for litterfall (positively correlated with MAP) and decomposition rate (positively correlated with MAT, subject to moisture availability) in DSF that further supports the theory of retarded heterotrophic soil respiration associated with lower MAT in the WC region.

The low detectability of fire regime effects (frequency and intensity combinations) on SOM contrasts with many other studies (Bennett et al., 2014; Wanthongchai et al., 2008; DeLuca and Sala, 2006; Adams and Attiwill, 1986). These studies claim to have examined fire frequency, however in these cases, fire frequency was confounded with substantial time since fire variation. The ability to infer fire regime effects beyond that of a single fire in these studies was therefore limited. Our finding of minimal effects of fire regimes with time since fire held relatively constant was consistent with other studies on soil carbon that have accounted for time since fire in evaluating fire frequency and fire intensity effects (e.g., Richards et al., 2012).

Higher estimated levels of % C_{Tot} under intermediate fire frequency in the WC and IW regions were possibly explained by the interplay between fire frequency, NPP and heterotrophic soil respiration, the latter two being driven by climate (Haverd et al., 2013; Penman and York, 2010; Lieth, 1975). Overall NPP is governed by precipitation and adequate growing season temperature (Lieth, 1975). Accumulation of C in the soil is determined by rates of aboveground material and root breakdown (inputs) and the rate of heterotrophic soil respiration, translocation and erosion (outputs) (Santín et al., 2016; Haverd et al., 2013), with the rate of heterotrophic soil respiration being most dependent on climate. In the WC and IW regions the limits to NPP are MAT and MAP respectively, with the precipitation effect being stronger (Haverd et al., 2013). Less frequent fire may lead to a drop in the availability of nutrients (e.g. phosphorus), elevating the C:N ratio in surface debris, retarding the rate of decomposition and mineralisation (Lagerström et al., 2009). This same limitation will apply to root productivity, although the nature of root material makes it more amenable to

incorporation into the soil SOM pool than litter (Jackson et al., 2017). The DSF ecosystem under study is dominated by resprouting species in all vegetation strata (Hammill et al., 2016), only the contribution of fire sensitive species' root mortality will have any effect on post fire SOM pool over the short term. Combined with the absence of fire this would slow the movement of aboveground material to the soil. In the highest fire frequency, aboveground material may be consumed at a rate faster than it could be incorporated into the soil. The intermediate frequency represents a situation where aboveground material has sufficient time to provide soil inputs (breakdown accelerated by second fire and higher nutrient availability) at a rate higher than heterotrophic soil respiration. Heterotrophic soil respiration is more strongly inhibited by lower MAT, which may lead to higher total C values in the WC region. In the WW region both NPP and heterotrophic soil respiration are potentially at the maximum for these soil types (Haverd et al., 2013). This has enabled soil C levels to fully recover between fires, even at the highest frequency treatment.

The peak in % C_{Tot} under the LH fire intensity combination may relate to low intensity fire killing but not consuming significant standing vegetation. This would have resulted in an elevated lignified tissue load on and above the soil surface. A subsequent high intensity fire is then likely to accelerate the charring and decomposition of this material enabling its incorporation into the mineral soil over the ten years prior to sampling. We estimated that a low intensity fire followed by a high intensity fire (LH) may enhance soil C storage by $2.9 \text{ Mg} \cdot \text{ha}^{-1}$ with 95% credible intervals from -11.4 to $18.5 \text{ Mg} \cdot \text{ha}^{-1}$, and a 0.66 probability of the effect being positive. The estimate was based on an approximate landscape average soil bulk density of $0.9 \text{ g} \cdot \text{cm}^{-3}$ (from authors' unpublished data) limited to the top 5 cm of soil. This was applied to the difference in soil C concentration in comparison to two successive low intensity fires (LL). The similarity in size of soil C pools for high intensity fire followed by either high or low intensity fire (i.e., HH & HL respectively) to LL implies a similar difference to LH. However, these results infer that any gains in the % C_{Tot} pool from the LH combination could be subsequently lost following a third fire, irrespective of intensity.

This study was concerned with the C incorporated into the mineral soil where it is most protected from chemical, physical and biological degradation (Lal, 2013). The work has been done on ridgetops where C deposited on the soil surface is highly vulnerable to relocation and consumption (Santín et al., 2016; Santín et al., 2013). The fire regime may produce PyC, some of which is RPC, but unless it is retained on the site in a protected location, such as mineral soil, its fate is largely indeterminate and therefore not amenable to manipulation via the fire regime.

4.1. Limitations

The well documented variability in the distribution of soil C in natural systems demands a high level of targeted replication (Lal, 2013). Acting against this is the cost and time required to collect, process and analyse large numbers of samples. Potential variation in the results presented here was minimised by using a stratified sampling strategy that targeted micro-sites where differences in total C were expected. This potentially maximises insights that could be gained from relatively limited replication. Greater replication of sites at the treatment level (i.e., climate, fire frequency and fire intensity) may have provided more precision in the estimation of the fire regime effects. Nonetheless, the magnitude of these effects is likely to remain relatively small even under higher levels of replication. Additionally, effects of variations on 40 years of recent fire history were examined but effects of prior fire regimes may be stored in the pool of soil C. It was assumed that longer-term effects prior to the limits of the recorded fire history were homogenised across the sites.

As Jenkins et al. (2016) have argued there is a need for a consistent definition of PyC. Accordingly, the term RPC was used here because only the most recalcitrant C was likely to have survived the digest process. It is important to recognize that all PyC analytical techniques have their limitations (Zimmerman and Mitra, 2017). In the case of the KMD method it is possible that some organic compounds such as waxes would survive this particular digest. Although we are confident that the presence of this material did not have a strong influence on our results, the possibility of uncertainties relating to variation in concentration of these materials in the matrix for the RPC values is acknowledged but not quantified. Reducing the soil and charcoal to a very fine fraction prior to digest may have made the SOM readily available for oxidation, though this was done consistently across the entire treatment range. In contrast, other studies have digested coarser material (e.g. fraction <2 mm) containing a wider spectrum of particle sizes with differing oxidation potential (Licata and Sanford, 2012; Ball et al., 2010; Kurth et al., 2006). In addition, these studies have used filtration to recover the soil residue, which introduces the potential for organic contamination. Strong capillary action on the finer organic matter was observed in this study during filtration attempts, which resulted in its loss. It was considered more prudent to minimise the spectrum of particle sizes and avoid contamination and other complications associated with filtration. As a result, a highly consistent and conservative estimation of PyC was produced that provided a relatively robust basis for investigation of the effects of climate, fire regime and site characteristics on the most recalcitrant fraction of soil C as well as total soil C.

Appendix A

Table A.1

Importance in ensemble modelling (% inclusion) of the predictors fire frequency, region and site characteristics and their interactions for predicting mean total % C. Fire frequency was represented as the number of fires (1,2,4) over 30 years prior to sampling 10 years post fire. Regions were characterized as: wet, cool (WC), annual precipitation 1200 mm and annual average temperature 12 °C; wet, warm (WW) annual precipitation 1200 mm, annual average temperature 16 °C; intermediate precipitation, warm (IW) annual precipitation 1000 mm, annual average temperature 16 °C. Soil depth was characterized by two layers (a, 0–5 cm; b, 5–15 cm). Micro sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open). Table includes the results of regression analysis, coefficient means, upper and lower bounds (95% confidence) and inclusion of zero or not within coefficient bounds (non-zero).

Effects	Variable	Percent inclusion	Mean included	Mean overall	Lower	Upper	Non-zero
Main	Intercept	100.00	0.3360	0.3360	0.2613	0.4109	1

(continued on next page)

4.2. Implications and future directions

Based on our findings, future work on the manipulation of soil C pools via alteration of the fire regime in natural ecosystems needs experiments designed to explore more than just time since fire or single fire effects. A comprehensive and cumulative assessment of fire regime effects is required. The costs of future studies in natural systems, such as these eucalypt forests, may be constrained through a much greater emphasis on measuring % C_{Tot} (relatively inexpensive analysis), based on the assumption that the recalcitrant C proportion and the inferred conversion rate is constant. This conclusion is further supported by the work of Lal (2013) who asserts that mean residence time for soil C is more driven by physical protection from erosion, consumption and abiotic factors unrelated to the chemical recalcitrance of the C in question.

This study suggests that potential for manipulating fire regimes to enhance soil C sequestration, or even minimising soil C losses, is limited in this forest type. Climate variations were more influential determinants of estimated total soil C pools than fire frequency or intensity factors. By considering the difference in mean % C_{Tot} between climate regions and converting to Mg·ha⁻¹ (using the same soil depth and bulk density applied to the differences in intensity combination, Section 4 above), a simple estimate of C stock shifts was generated. Climate shifts towards warmer MAT by up to 4 °C (which is within the scope of fifty year projections by climate models: CSIRO and Bureau of Meteorology, 2015) have the potential to substantially lower soil C pools in the WC region (ca. 4.5 Mg·ha⁻¹). If temperature increases by this amount and MAP decreases by 200 mm, the predicted losses of C to the atmosphere are in the order of 7.3 Mg·ha⁻¹. In the WC region, there was considerably more C in sites that had experienced 2 fires in 40 years compared to 4 fires in 40 years. Increasing fire frequency may therefore further exacerbate C losses in this region.

A whole ecosystem, fire regime and climate C model, incorporating existing data on landscape factors such as soil cover, tree cover and estimations of above and belowground biomass can be developed on this basis, enabling an integrated understanding of fire regime and climate effects on the total C stores. Such an understanding is required to predict the consequences of landscape management decisions, in conjunction with future climate changes, on terrestrial C stores in these flammable ecosystems.

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Table A.1 (continued)

Effects	Variable	Percent inclusion	Mean included	Mean overall	Lower	Upper	Non-zero
Main	regionCodeWC	100.00	0.1975	0.1975	0.0998	0.2998	1
Main	depthb_5to15	100.00	−0.3223	−0.3223	−0.3935	−0.2457	1
Main	barkR	100.00	0.0985	0.0985	0.0063	0.1850	1
Main	barkS	100.00	0.0930	0.0930	−0.0074	0.1800	0
Main	regionCodeWW	99.59	0.0971	0.0967	−0.0086	0.1914	0
Main	firefreq2	96.72	0.0662	0.0640	−0.0432	0.1755	0
2 way	regionCodeWW:firefreq2	90.72	−0.1368	−0.1241	−0.2609	−0.0151	1
2 way	firefreq2:barkR	84.27	0.0886	0.0747	−0.0328	0.2085	0
Main	firefreq4	79.18	0.0258	0.0205	−0.0696	0.1150	0
2 way	regionCodeWC:firefreq2	74.65	−0.0826	−0.0616	−0.2229	0.0554	0
2 way	regionCodeWC:barkS	74.40	0.0763	0.0568	−0.0460	0.1959	0
2 way	firefreq2:barkS	72.45	0.1015	0.0736	−0.0149	0.2218	0
2 way	regionCodeWC:firefreq4	66.30	−0.1095	−0.0726	−0.2414	0.0195	0
2 way	regionCodeWC:barkR	64.72	0.0323	0.0209	−0.0954	0.1592	0
2 way	depthb_5to15:firefreq2	62.40	−0.0740	−0.0462	−0.1734	0.0224	0
2 way	regionCodeWW:barkR	54.05	0.0569	0.0308	−0.0559	0.1696	0
2 way	regionCodeWW:depthb_5to15	52.20	−0.0518	−0.0271	−0.1470	0.0452	0
2 way	regionCodeWW:barkS	49.00	0.0529	0.0259	−0.0661	0.1734	0
2 way	firefreq4:barkS	48.84	−0.0086	−0.0042	−0.1311	0.1102	0
2 way	regionCodeWC:depthb_5to15	48.65	0.0480	0.0234	−0.0476	0.1450	0
2 way	regionCodeWW:firefreq4	47.20	0.0797	0.0376	−0.0391	0.2029	0
3 way	regionCodeWC:firefreq2:barkR	46.28	0.1205	0.0558	−0.0438	0.2990	0
2 way	firefreq4:barkR	39.41	−0.0189	−0.0075	−0.1387	0.0992	0
3 way	regionCodeWC:firefreq2:barkS	38.03	−0.0727	−0.0277	−0.2432	0.0939	0
3 way	regionCodeWC:firefreq4:barkS	34.71	0.1061	0.0368	−0.0669	0.2744	0
3 way	regionCodeWW:firefreq2:barkR	33.97	−0.0239	−0.0081	−0.1888	0.1439	0
2 way	depthb_5to15:firefreq4	32.51	0.0031	0.0010	−0.0986	0.1037	0
3 way	regionCodeWW:depthb_5to15:firefreq2	30.20	0.0449	0.0136	−0.0971	0.2057	0
3 way	regionCodeWW:firefreq2:barkS	27.70	−0.0113	−0.0031	−0.1755	0.1572	0
3 way	regionCodeWC:firefreq4:barkR	21.28	−0.0570	−0.0121	−0.2161	0.1188	0
3 way	regionCodeWC:depthb_5to15:firefreq2	19.88	−0.0105	−0.0021	−0.1551	0.1474	0
3 way	regionCodeWW:firefreq4:barkS	18.55	−0.0686	−0.0127	−0.2282	0.0949	0
3 way	regionCodeWW:firefreq4:barkR	15.61	−0.0115	−0.0018	−0.1737	0.1456	0
3 way	regionCodeWC:depthb_5to15:firefreq4	14.53	−0.0169	−0.0025	−0.1621	0.1290	0
3 way	regionCodeWW:depthb_5to15:firefreq4	13.45	−0.0057	−0.0008	−0.1544	0.1386	0

Table A.2

Importance in ensemble modelling (% inclusion) of the predictors fire intensity and site characteristics and their interactions for predicting mean total % C. Fire intensity was rated low (L), tree crowns unaffected, or high (H), tree crowns affected. Sites selected from two successive fires separated by 8 years, soil collected 10 years post fire, yielding four intensity combinations (LL, LH, HL, HH). Soil depth was characterized by two layers (a, 0–5 cm; b, 5–15 cm). Micro sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open). Table includes the results of regression analysis, coefficient means, upper and lower bounds (95% confidence) and inclusion of zero or not within coefficient bounds (non-zero).

Effect	Variable	Percent inclusion	Mean included	Mean overall	Lower	Upper	Non-zero
	Intercept	100.00	0.3297	0.3297	0.2460	0.4209	1
Main	depthb_5to15	100.00	−0.3120	−0.3120	−0.3886	−0.2235	1
Main	barkS	99.77	0.1872	0.1867	0.0944	0.2883	1
Main	barkR	92.29	0.0980	0.0904	0.0039	0.1834	1
Main	intensityLH	90.72	0.1180	0.1071	0.0176	0.2296	1
2 way	depthb_5to15:intensityLH	53.70	−0.0842	−0.0452	−0.2052	0.0396	0
Main	intensityHL	52.70	−0.0285	−0.0150	−0.1459	0.0901	0
Main	intensityHH	52.15	−0.0293	−0.0153	−0.1385	0.0811	0
2 way	intensityLH:barkS	49.24	−0.0787	−0.0388	−0.2246	0.0542	0
2 way	intensityLH:barkR	33.90	−0.0191	−0.0065	−0.1675	0.1287	0
2 way	intensityHL:barkR	31.33	0.0861	0.0270	−0.0640	0.2428	0
2 way	intensityHH:barkR	27.25	0.0625	0.0170	−0.0890	0.2070	0
2 way	intensityHH:barkS	25.17	−0.0436	−0.0110	−0.1990	0.1059	0
2 way	intensityHL:barkS	24.35	0.0003	0.0001	−0.1662	0.1575	0
2 way	depthb_5to15:intensityHL	23.80	−0.0311	−0.0074	−0.1747	0.1111	0
2 way	depthb_5to15:intensityHH	22.15	0.0032	0.0007	−0.1395	0.1447	0

Table A.3

Importance in ensemble modelling (% inclusion) of the predictors fire frequency, region and site characteristics and their interactions for predicting mean % RPC. Fire frequency was represented as the number of fires (1,2,4) over 30 years prior to sampling 10 years post fire. Regions were characterized as: wet, cool (WC), annual precipitation 1200 mm and annual average temperature 12 °C; wet, warm (WW) annual precipitation 1200 mm, annual average temperature 16 °C; intermediate precipitation, warm (IW) annual precipitation 1000 mm, annual average temperature 16 °C. Soil depth was characterized by two layers (a, 0–5 cm; b, 5–15 cm). Micro sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open). Table includes the results of regression analysis, coefficient means, upper and lower bounds (95% confidence) and inclusion of zero or not within coefficient bounds (non-zero).

Variable	Percent inclusion	Mean included	Mean overall	Lower	Upper	Non-zero
Intercept	100.00	−1.0194	−1.0194	−1.0922	−0.9580	1
logInitC	100.00	1.0582	1.0582	0.9364	1.2012	1
depthb_5to15	16.73	0.0501	0.0084	−0.0749	0.1636	0
logInitC:depthb_5to15	16.23	−0.3350	−0.0544	−0.6161	−0.0591	1
firefreq4	13.72	0.0735	0.0101	0.0028	0.1325	1
regionCodeWC	3.97	−0.1467	−0.0058	−0.2956	−0.0108	1
logInitC:regionCodeWC	3.47	0.2511	0.0087	0.0235	0.4790	1
regionCodeWW	1.67	−0.0644	−0.0011	−0.1903	0.0244	0
regionCodeWC:firefreq4	1.64	0.1422	0.0023	0.0376	0.2528	1
barkR	1.58	0.0552	0.0009	0.0034	0.1081	1
logInitC:regionCodeWW	0.82	0.1388	0.0011	−0.1048	0.4487	0
barkS	0.43	−0.0405	−0.0002	−0.0953	0.0007	0
regionCodeWW:firefreq4	0.42	0.0757	0.0003	−0.0684	0.2030	0
depthb_5to15:firefreq4	0.42	0.0596	0.0003	−0.0506	0.1520	0
firefreq2	0.28	−0.0159	0.0000	−0.0615	0.0191	0
regionCodeWC:depthb_5to15	0.19	−0.0787	−0.0001	−0.1772	0.0193	0
firefreq4:barkR	0.18	−0.0103	0.0000	−0.1024	0.1255	0
logInitC:regionCodeWC:depthb_5to15	0.17	0.2554	0.0004	−0.1225	0.5529	0
regionCodeWW:depthb_5to15	0.10	0.0624	0.0001	−0.0855	0.2323	0

Table A.4

Importance in ensemble modelling (% inclusion) of the predictors fire intensity and site characteristics and their interactions for predicting mean % RPC. Fire intensity was rated low (L), tree crowns unaffected, or high (H), tree crowns affected. Sites selected from two successive fires separated by 8 years, soil collected 10 years post fire, yielding four intensity combinations (LL, LH, HL, HH). Soil depth was characterized by two layers (a, 0–5 cm; b, 5–15 cm). Micro sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open). Table includes the results of regression analysis, coefficient means, upper and lower bounds (95% confidence) and inclusion of zero or not within coefficient bounds (non-zero).

Variable	Percent inclusion	Mean included	Mean overall	Lower	Upper	Non-zero
Intercept	100.00	−0.9919	−0.9919	−1.0438	−0.9425	1
logInitC	100.00	1.1529	1.1529	1.0239	1.2888	1
barkS	4.38	−0.0703	−0.0031	−0.1356	−0.0110	1
depthb_5to15	2.70	0.0531	0.0014	−0.0577	0.1928	0
logInitC:depthb_5to15	2.14	−0.3075	−0.0066	−0.6345	0.0594	0
intensityLH	0.73	0.0278	0.0002	−0.0488	0.0845	0
intensityHH	0.52	0.0160	0.0001	−0.0395	0.0870	0
barkR	0.45	0.0098	0.0000	−0.0695	0.0888	0
intensityHL	0.41	0.0003	0.0000	−0.0624	0.0514	0
intensityLH:barkS	0.10	−0.0975	−0.0001	−0.1868	−0.0053	1

Appendix B

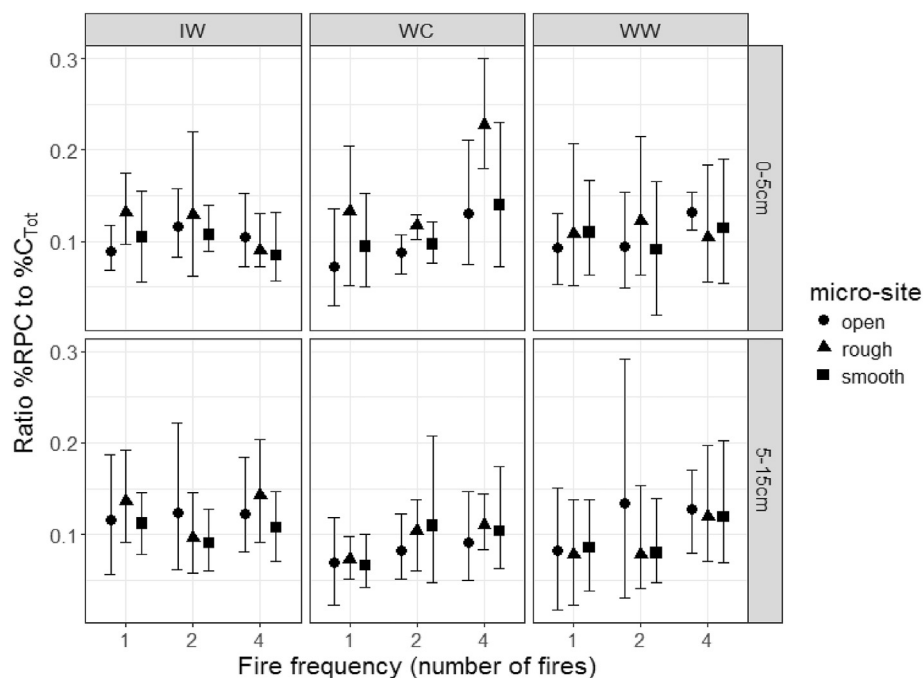


Fig. B.1. Ratio of %RPC to %C_{Tot} in relation to fire frequency, region and site characteristics shown as mean values (points) with 95% credible intervals. Within each combination, mean %RPC was calculated for each field sample using a Bayesian procedure in which a heavy-tailed distribution (t-distribution with flexible shape parameter) was fitted to five replicate analytical values. This approach ensured that any outlying analytical values for a field sample would be down-weighted in a data-driven manner. Markov Chain Monte Carlo estimation resulted in a set of credible mean %RPC values for each field sample and a corresponding set of ratio values to %C_{Tot} from which the means and intervals shown here were derived. Fire frequency was represented as the number of fires (1,2,4) over 30 years prior to sampling 10 years post fire. Regions were characterized as: wet, cool (WC), annual precipitation 1200 mm and annual average temperature 12 °C; wet, warm (WW) annual precipitation 1200 mm, annual average temperature 16 °C; intermediate precipitation, warm (IW) annual precipitation 1000 mm, annual average temperature 16 °C. Soil depth was characterized by two layers (0–5 cm; 5–15 cm). Micro sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open).

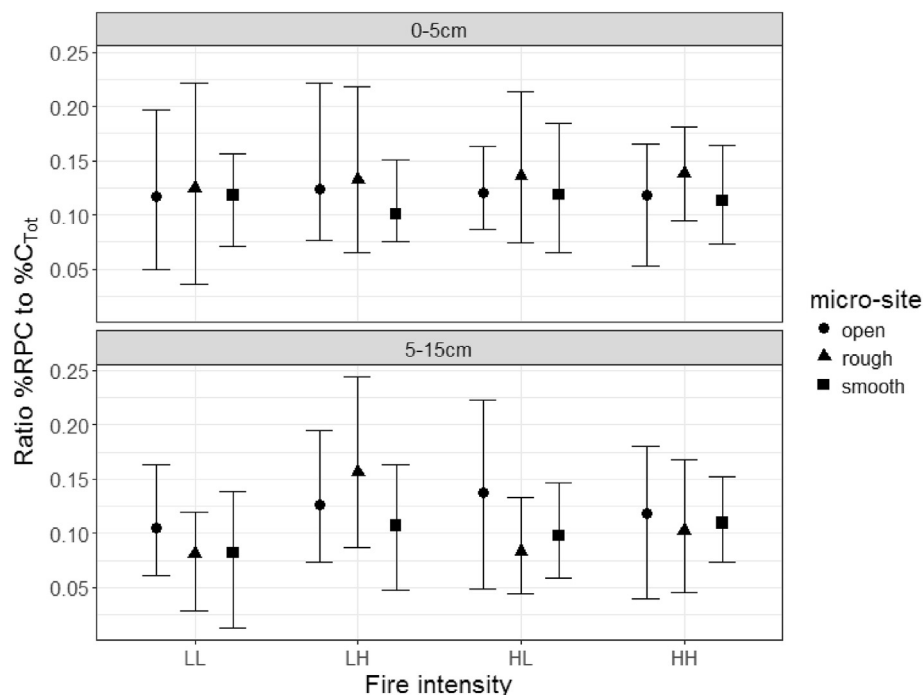


Fig. B.2. Ratio of %RPC to %C_{Tot} in relation to fire intensity and site characteristics shown as mean values (points) with 95% credible intervals. Within each combination, mean %RPC was calculated for each field sample using a Bayesian procedure in which a heavy-tailed distribution (t-distribution with flexible shape parameter) was fitted to five replicate analytical values. This approach ensured that any outlying analytical values for a field sample would be down-weighted in a data-driven manner. Markov Chain Monte Carlo estimation resulted in a set of credible mean %RPC values for each field sample and a corresponding set of ratio values to %C_{Tot} from which the means and intervals shown here were derived. Fire intensity was rated low (L), tree crowns unaffected, or high (H), tree crowns affected. Sites selected from two successive fires separated by 8 years, soil collected 10 years post fire, yielding four intensity combinations (LL, LH, HL, HH). Soil depth was characterized by two layers (0–5 cm; 5–15 cm). Micro sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open).

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